Indonesian Journal of Biology Education
Vol. 3, No. 1, 2020, pp: 24-30
pISSN: 2654-5950, eISSN: 2654-9190
Email: ijobe@untidar.ac.id
Website: jurnal.untidar.ac.id/index.php/ijobe

Molecular Identification of Two Rare Actinomycetes Isolated from Mosul, Iraq

Talal S. Salih[1*], Mohammed A. Ibraheem[2], Muhammad A. Muhammad[2]
[1]Department of Biophysics, College of Science, University of Mosul
[2] Department of Biology, College of Science, University of Mosul
Email: [1]talal.salih@uomosul.edu.iq,
[2]mohrsbio122@uomosul.edu.iq,
[3]mohamadmosto0@gmail.com,

Article History

Received :11 – 05 – 2020
Revised : 10 – 06 – 2020
Accepted : 16 – 07 – 2020

***Corresponding Author**
Talal S. Salih
Department of Biophysics
University of Mosul
00964-Mosul, Iraq
talal.salih@uomosul.edu.iq

Keywords:
rare actinomycetes, *Actinoplanes* sp. MOSUL, *Amycolatopsis* sp. MOSUL, 16S rRNA gene.

Abstract

Rare actinomycetes from diverse habitats are continued to be isolated and screened for their novel bioactive compounds. The present study aims to molecular, morphological and physiological characterisation of two rare actinomycetes isolated from an Iraqi soil. Based on the 16S rRNA gene sequencing, the two isolates were categorized into two different rare genera Actinoplanes and Amycolatopsis that were designated as Actinoplanes sp. MOSUL and Amycolatopsis sp. MOSUL respectively. Phylogenetic trees analyses revealed that Act. sp. MOSUL was closely related strain to Act. xinjiangensis (jgi.1107663; identity 96.75%) and Act. lobatus (AB037006; identity 96.76%), and Amy. sp. MOSUL was most related to Amy. bullii (HQ65173099; identity 99.71%) and Amy. tolypomycina (FNSO01000004; identity 99.26%). The two rare isolates had different morphological properties when grown on International Streptomyces Project (ISP) media, and different physiological and biochemical patterns when grown on Minimal Medium (MM) agar alone or supplemented with different carbon or chemical sources. The ribosomal 16S gene sequences of both Act. sp. MOSUL and Amy. sp. MOSUL strains were submitted to the National Center for Biotechnology Information (NCBI) and deposited in the GenBank database under the accession numbers MN095717 and MN095769 respectively.

1. INTRODUCTION

Actinomycetes are Gram-positive aerobic bacteria. Compared with other bacteria, they have large linear genomes and high guanine and cytosine (GC) content, which form an extensive branched mycelium fragmenting into non-motile spore chains (Kämpfer, 2006). They are prevalent in soils, where they play a vital role in the cycle of organic carbon substances. They constitute about 20 per cent of the microbial flora in the soil and their diversity often depends on environmental conditions (Barka et al., 2016).

Actinomycetes are renowned for being a rich source of bioactive chemical compounds with great potential for medical and pharmaceutical applications. Although the isolation of terrestrial actinomycetes has been started extensively in the early 1950s, researchers from all around the word are continuing of isolating and discovering of new and rare actinomycetes (Qin et al., 2009; Kruasuwan et al., 2017; Singh et al., 2018; Benhadj et al., 2019; Banoon et al., 2019) for novel pharmaceutical compounds and for combating human pathogens.

Rare actinomycetes are described as actinobacteria in which the isolation rate using traditional methods is lower than the genus of *Streptomyces* such as *Actinoplanes*, *Amycolatopsis*, *Actinomadura*, *Dactylosporangium*, *Kibdelosporangium*, *Kitasatospora*, *Microbiospora*, *Planomonospora*, *Planobispora*, *Salinispora*, *Streptosporangium*, and *Verrucosispora* (Lazzarini et al., 2000). The number of genera of rare actinomycetes has further been increased (Tiwari and Kupta, 2013).

The genus *Actinoplane*s was first established by Couch (1950). The type species of the genus *Actinoplanes* is *Act. philippinensis* Couch 1950 and currently includes 52 species with valid published names (Parte, 2018). Otherwise, the genus *Amycolatopsis* was described by Lechevalier et al. (1986) and the type species of the genus *Amycolatopsis* is *Amy. orientalis*. The genus

consists of 87 recognised species and 4 subspecies (Parte, 2018), most of which have been isolated from terrestrial environment sites during the last few years using a range of phenotypic and genotypic characteristics (Zucchi et al., 2012; Klykleung et al., 2015; Jamjan et al., 2018; Peng et al., 2019).

This study was carried out in order to isolate rare actinomycetes from Iraqi soils and to conduct the methodology of the ribosomal 16S rRNA gene for molecular identification to species level and to elucidate their morphological, biochemical and physiological features.

2. RESEARCH METHODS

a. Selective Isolation

Soil samples at depth of 10 cm were aseptically collected from grassland area belonging to Mosul University campus located in Mosul city, Iraq. The soil samples were smashed, thoroughly mixed and large debris removed to obtain fine particles from the soil. For *Act.* sp. MOSUL and *Amy*. sp. MOSUL isolation, the soil samples were treated with dry heat treatment at 120°C for one hour. The heated samples (1.0 g) were then treated with sterile powdered calcium carbonate (10:1 w/w), diluted to 10^{-3} with sterile water and were spread-plated on selective SM1medium which was prepared as described by Tan et al. (2006). The plates were incubated for up to 21 days at 28°C. The putative isolates of the genus *Actinoplanes* and *Amycolatopsis* have been purified and maintained on the maltose yeast extract (MYM) medium (Shepherd et al., 2010).

b. Morphological and Physiological Characterisation

Characterisation of *Act*. sp. MOSUL and *Amy*. sp. MOSUL was conducted using the International Streptomyces Project (ISP) standardized methods (Shirling & Gottlieb, 1966). The selected ISP media were Yeast Malt Agar (ISP-2), Oatmeal Agar (ISP-3), Inorganic Salt Starch Agar (ISP-4) and Glycerol Asparagine Agar Base (ISP-5). Production of melanin was evaluated using the media ISP-6 and ISP-7. The strain descriptions were categorized as growth, substratum colour and aerial mycelium, and soluble pigment colour produced. Colour was described using classic code RAL K7 (edition 2012, Germany). Minimal Medium (MM) agar (Kieser et al., 2000) supplemented with glucose (10g/L) as the carbon source was prepared for NaCl test. A stock MM medium of 10% NaCl was also prepared; the stock was mixed with 0% MM medium to give concentrations of 0, 1, 2.5, 5, 7.5 and 10% NaCl. Both pH and temperature tolerance assays were assessed on ISP-2 medium. ISP-2 medium was adjusted to pH 2, 4, 6, 8, 10, and 12 and at 5, 10, 15, 20, 25, 30, 37, 40 and 45 °C. The decomposition of urea on Christensen's urea agar containing 2% urea was determined according to Gordon et al. (1974). Degradation of casein (0.5% final concentration) was determined using MM medium. Catalase activity was detected by dropping 3% of H_2O_2 on well-developed agar plate colonies and observing any gas bubbles produced. Plates were poured out with prepared medium in each assay. Upon inoculation with *Act.* sp. MOSUL or *Amy*. sp. MOSUL, plates were incubated for 21days at 30°C. Carbon source utilization tests were determined on MM agar medium supplemented with different carbon sources.

c. DNA extraction, 16S Gene Amplification, Sequencing and Phylogenetic Trees Construction

The *Act.* sp. MOSUL and *Amy*. sp. MOSUL were grown for 7 days at 30°C on a rotary shaker (250 rpm) in a 50 ml falcon tube containing 20 ml of Tryptic Soy Broth (TSB) medium. The procedure of Wizard® Genomic DNA Purification Kit (Promega, USA) was then followed for DNA extraction. Polymerase chain reaction (PCR) of 16S rRNA genes amplification from genomic DNA of both *Act*. sp. MOSUL and *Amy*. sp. MOSUL isolates were performed following the protocol of Promega PCR Master Mix. For each PCR reaction 1µl of DNA template, 1µl of 10µM of each conserved primers 27F (5´-AGAGTTTGATCCTGGCTCAG-3´) and 1492R (5´-TACGGCTACCTTGTTACGACTT-3´) (Goodfellow et al., 2007), 25µl PCR Master Mix (2X) and 22µl ddH_2O for a total volume of 50µl. The PCR amplification cycle consisted of initial denaturation step at 95°C for 5 min followed by 35 amplification cycles of 95°C for 45 seconds for denaturation, 55°C for 1 min for primers annealing, 72°C for 1 min for primers extension, and ending with a final extension step at 72°C for 10 min. The PCR products along with the primers used in this study were sent to Germany (Eurofins MWG, Ebersberg, Germany) for sequencing. The obtained 16S gene sequences of *Act*. sp. MOSUL and *Amy*. sp. MOSUL were searched with sequences of closely related species of the genera *Actinoplanes* and *Amycolatopsis* in the EzTaxon specialized ribosomal database (Kim et al., 2012). Sequences shared the highest similarity with both *Act*. sp. MOSUL and *Amy*. sp. MOSUL were retrieved and downloaded in FASTA format. A maximum-likelihood (Felsenstein, 1981) phylogenetic tree for each 16S gene of *Act.* sp. MOSUL and *Amy*. sp. MOSUL were constructed using the program MEGA version 7.0 (Tamura et al., 2013) from the aligned sequences using Multiple Sequence Comparison by Log Expectation (MUSCLE) software (Edgar, 2004).

3. RESULTS AND DISCUSSION

a. Cultural Characteristics

The cultivation and phenotypic characterization of rare actinomycetes have been studies by many researchers on the bases of utilizing their ability to grow on different cultural media (Bredholdt et al., 2007; Tiwari and Kupta, 2013; Aziz et al., 2019). It was observed that *Act.* sp. MOSUL grew abundantly on ISP-2 and ISP-3 media while the growth was found to be poor on ISP-4 and ISP-5 media. The mycelia colour was varied according to the ISP-medium and the melanin pigment was not produced (Table 1). Good growth of *Amy*. sp. MOSUL strain was observed on ISP-2 and ISP-3 media, and moderate growth on ISP-4 and ISP-5 media, and the melanin pigment was produced on both ISP-6 and ISP-7 (Table 1). Studies have stated that ISP-media were especially valuable to differentiate the new actinomycetes isolates, and helpful for colour determination and for melanin pigment production (Jaradat et al., 2008; Sun et al., 2009; Ara et al., 2010; Klykleung et al., 2015).

Table 1. Culture Characteristics *of Act.* sp. MOSUL and *Amy*. sp. MOSUL on ISP Media

	ISP-2	ISP-3	ISP-4	IS-P5	ISP-6/ISP-7
Act. sp. MOSUL	G: Abundant A: Salmon orange (RAL 2012) S: None	G: Abundant A: Signal orange (RAL 2010) S: None	G: Poor A: Signal orange (RAL 2010) S: None	G: Poor A: Pastel orang (RAL 2003) S: None	M: None produced
Amy. sp. MOSUL	G: Abundant A: Grey white (RAL 9002) S: None	G: Abundant A: Papyrus white (RAL 9018) S: None	G: Moderate A: Papyrus white (RAL 9018) S: None	G: Moderate A: Telegrey 4 (RAL 7047) S: None	M: Produced

G, Growth; A, Aerial mass colour; S, Soluble pigment, M, Melanin pigment

b. Physiological and Biochemical Characteristics

The physiological and biochemical of *Act.* sp. MOSUL and *Amy*. sp. MOSUL showed a range of different phenotypic properties that were similar to the results reported by other researchers (Kämpfer et al., 2007; Sun et al., 2009; Tang et al., 2010; Nie et al., 2012; Souza et al., 2015) describing these two genera. It was found that *Act.* sp. MOSUL tolerant to NaCl in concentrations up to 4%. The strain was found to grow well between the pH 4 to pH 8 range, and the growth was inhibited at 45ºC (Table 2). Decomposition of urea and catalase activity was positive, and casein degradation was negative. The strain could use L-Arabinose, D-Fructose, D-Galactose, D-Sucrose, D-Glucose and D-Xylose, but could not use D-Maltose and D-Manitol (Table 2 & 3). On the other hand, *Amy*. sp. MOSUL could grow in the presence of up to 8% NaCl, up to 75 µg/ml, at pH 8 and up to 40 ºC (Table 2). Decomposition of urea and catalase activity, and casein degradation were negative. The strain could use Arabinose, D-Fructose, D-Galactose, D-Sucrose, D-Glucose, D-Maltose and D-Xylose as sole carbon source, but could not use D-Manitol (Table 1 & 2).

Table 2. Physiological and Biochemical Characteristics of *Act.* sp. MOSUL and *Amy*. sp. MOSUL Strains

	Character	***Act.* sp. MOSUL**	***Amy*. sp. MOSUL**
Growth at NaCl (%)	**2**	+	+
	4	+	+
	6	−	+
	8	−	+
Growth at pH	**4**	+	+
	6	+	+
	8	+	+
	10	−	−
Growth at	**30 ºC**	+	+
	37 ºC	+	+
	40 ºC	+	+
	45 ºC	−	−
Urea decomposition	**2%**	+	−
Casein degradation	**0.5%**	−	−
Catalase activity	**3% H_2O_2**	+	−

Table 3. Growth Pattern of the *Act.* sp. MOSUL and *Amy.* sp. MOSUL in Different Carbon Sources

Carbon source	*Act.* sp. MOSUL	*Amy.* sp. MOSUL
L-Arabinose	+	+
D-Fructose	+	+
D-Galactose	+	+
D-Sucrose	+	+
D-Glucose	+	+
D-Maltose	−	+
D-Manitol	−	−
D-Xylose	+	+

c. Nucleotide Sequence Accession Numbers

The two rare strains designated as *Actinoplanes* sp. MOSUL and *Amycolatopsis* sp. MOSUL and their ribosomal 16S gene sequences were deposited in the NCBI Genbank database under the accession numbers MN095717 and MN095769 respectively.

d. Molecular Phylogeny Analysis

A neighbor-joining tree based on the highest 24 similar 16S rRNA gene sequences showed that the *Act*. sp. MOSUL (MN095717) strain is the most closely related strain to *Act. xinjiangensis* (jgi.1107663), *Act. lobatus* (AB037006), *Act. auranticolor* (U58526), *Act. sichuanensis* (EU531458), *Act. philippinensis* (jgi.1085831), *Act. subglobosus* (KM396265), *Act. hulinensis* (JQ073723), *Act. campanulatus* (AB036995) and *Act. capillaceus* (AB013495) with identity of 96.75%, 96.76%, 96.45%, 96.66%, 96.36%, 96.66%, 96.45%, 96.15% and 96.66% respectively (Figure 1) . *Act. xinjiangensis* and *Act. sichuanensis* were isolated from two soil sites in China. Interestingly, these strains exhibited strong inhibitory activity against some of Gram negative and positive mutltidrugs resistant bacteria (Sun et al., 2009). Thus, our isolated *Act*. sp. MOSUL strain might have a potent promising biological activity against clinical bacteria. While *Act. lobatus, Act. auranticolor* and *Act. campanulatus* were species of the genus *Actinoplanes* firstly isolated by Couch (1950), *Act. subglobosus*, *Act. hulinensis* and *Act. capillaceus* were isolated from different regions in Asia, Thailand, China and Japan respectively (Ngaemthao *et al*., 2016; Shen, *2013*; Matsumoto et al., 2000).

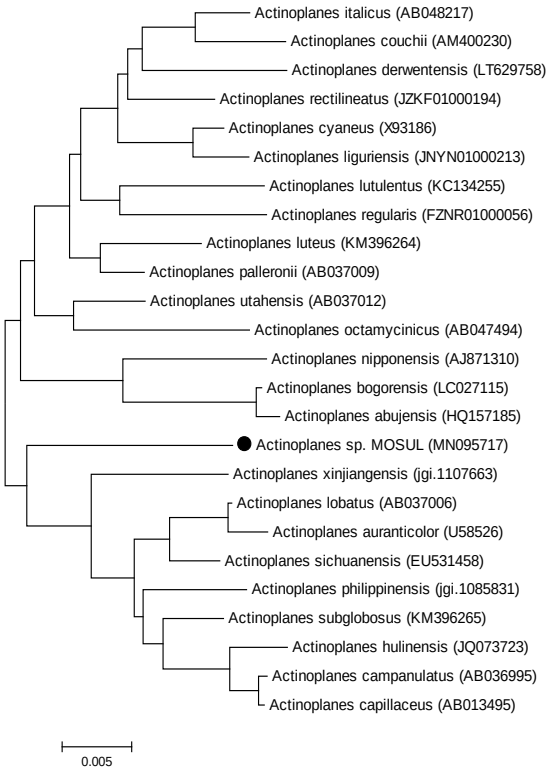

Figure 1. Neighbor-joining tree (1000X bootstrap) based on partial 16S rRNA gene sequences showing the relationshipa between *Actinoplanes* sp. MOSUL strain (indicated by black circle) and the most related 24 type strain species of the genus *Actinoplanes* that obtained from the EzTaxon database. Accession numbers are given in parentheses. Bar, 0.005 substitutions per nucleotide position.

On the other hand, *Amy. bullii* (HQ651730), *Amy. tolypomycina* (FNSO01000004), *Amy. plumensis* (AY262825), *Amy. eburnea* (MH598363) and *Amy. rhabdoformis* (KF779477) were the most closely related strains of the highest 23 similar strains to *Amy*. sp. MOSUL (MN095769) with identity of 99.71%, 99.26%, 98.46%, 98.32% and 98.74% respectively (Figure 2). *Amy. bullii* was isolated from an arid Australian soil sample (Zucchi *et al*., 2012). *Amy. tolypomycina* which showed activity against methicillin resistant staphylococci was isolated from an Indian soil sample (Wink et al., 2003). *Amy. plumensis*, *Amy. eburnean* and *Amy. rhabdoformis* were isolated from different environmental samples from of the main island of New Caledonia, Thailand and Brazil respectively (Saintpierre-Bonaccio *et al*,, 2005; Chaiya et al., 2019; Souza et al., 2015).

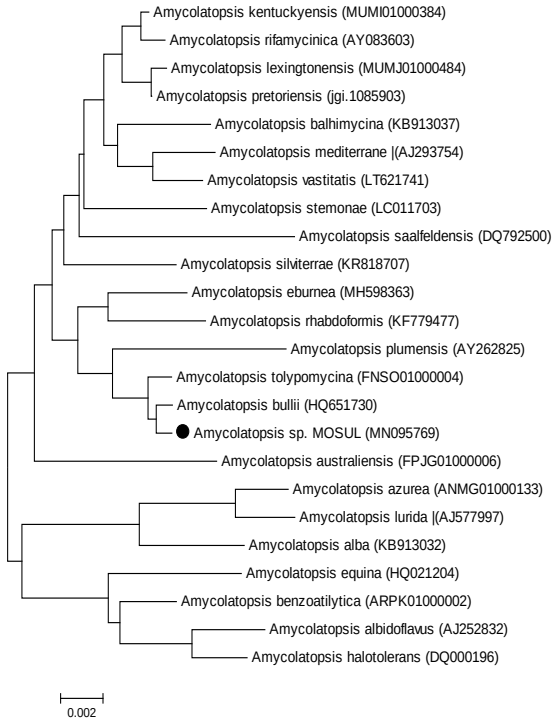

Figure 2. Neighbor-joining tree (1000X bootstrap) based on partial 16S rRNA gene sequences showing the relationshipa between *Amycolatopsis* sp. MOSUL strain (indicated by black circle) and the most related 23 type strain species of the genus *Actinoplanes* that obtained from the EzTaxon database. Accession numbers are given in parentheses. Bar, 0.002 substitutions per nucleotide position.

4. CONCLUSIONS AND RECOMMENDATIONS

Actinomycetes are well-known to be an important source of useful secondary metabolites and new antibiotics. In this study, two new rare actinomycetes designated as *Act.* sp. MOSUL and *Amy.* sp. MOSUL from Iraqi soils were isolated and identified. The 16S rRNA gene sequences of these two strains were submitted to the NCBI Genbank database and deposited under the accession numbers MN095717 and MN095769 respectively. The two isolates displayed different patterns of morphology and physiology when grown on different cultural media supplemented with different chemical sources. The phylogenetic trees analysis put these strains close to strains that are well-known to produce significant active metabolites against some of Gram negative and positive bacteria. Our findings encourage the exploration of isolation and identification further new rare actinomycetes from the Iraqi soils as well as carrying advance analyses that may lead to finding new drug targets. .

ACKNOWLEDGEMENT

The authors are very grateful to the University of Mosul and the College of Science for their provided facilities, which helped to improve the quality of this work.

REFERENCES

Albdaiwi, R. N., Khyami-Horani, H. & Ayad, J. Y. (2019). Plant Growth-Promoting Rhizobacteria: An Emerging Method for the Enhancement of Wheat Tolerance against Salinity Stress-(Review). *Jordan Journal of Biological Sciences*, *12***,** 525-534.

Ara, I., Yamamura, H., Tsetseg, B., Daram, D. & Ando, K. (2010). *Actinoplanes toevensis* sp. nov. and *Actinoplanes tereljensis* sp. nov., isolated from Mongolian soil. *Int J Syst Evol Microbiol, 60*, 919-27.

Aziz, Z. S., Banoon, S. R. & Hassain, A. S. (2019). Molecular Detection of Some Virulence and Antibiotic Resistance Genes in *Staphylococcus aureus* Strains Isolated from Bovine Mastitis in Maysan Province. *ARCTIC Journal*, *72*, 58-76.

Banoon, S. R., Kadhim, Z. K, Aziz, Z. S. & EWadh, R. M. (2019). Using Random Amplified Polymorphic DNA (RAPD) Fingerprinting Technique to Analyze Genetic Variation in *Staphylococcus aureus* Isolated from Different Sources in Babylon Province Hospitals. *Indian Journal of Public Health Research & Development*, *10*, 1300-1305.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C. , Meier-Kolthoff, J. P., Klenk, H. P., Clément, C., Ouhdouch, Y. & van Wezel, G. P. (2016). Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, *80*, 1-43.

Benhadj, M., Gacemi-Kirane, D., Menasria, T., Guebla, K. & Ahmane, Z. (2019). Screening of rare actinomycetes isolated from natural wetland ecosystem (Fetzara Lake, northeastern Algeria) for hydrolytic enzymes and antimicrobial activities. *Journal of King Saud University Science*, *31*, 706-712.

Bredholdt, H., Galatenko, O. A., Engelhardt, K., Tjaervik, E., Terekhova, L. P. & Zotchev, S. B. (2007). Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity and biological activity. *Environ Microbiol*, *9*, 2756-2764.

Chaiya, L., Matsumoto, A., Wink, J., Inahashi, Y., Risdian, C., Pathom-Aree, W. & Lumyong S. (2019). *Amycolatopsis eburnea* sp. nov., an actinomycete associated with arbuscular

mycorrhizal fungal spores. *International journal of systematic and evolutionary microbiology*, 69, 3603-3608.

Couch, J. N. (1950). Actinoplanes, a new genus of the Actinomycetales. *Journal of the Elisha Mitchell Scientific Society, 66,* 87-92.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution, 17,* 368–376.

Goodfellow, M., Kumar, Y., Labeda, D. P. & Sembiring L. (2007). *The Streptomyces violaceusniger* clade: a home for Streptomycetes with rugose ornamented spores. *Antonie van Leeuwenhoek, 92,* 173-199.

Gordon, R. E., Barnett, D. A., Handerhan, J. E & Pang CH. (1974). *Nocardia coeliaca, Nocardia autotrophica,* and the nocardin strain. *International Journal of Systematic and Evolutionary Microbiology, 24,* 54–63.

Jamjan, W., Suriyachadkun, C., Tanasupawat, S., Sakai, K., Tashiro, Y., Okugawa, Y. & Tongpim, S. (2018). *Amycolatopsis silviterrae* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol, 68,* 1455-1460.

Jaradat, Z., Dawagreh, A., Ababneh, Q. & Saadoun, I. (2008). Influence of Culture Conditions on Cellulase Production by *Streptomyces* Sp. (Strain J2). *Jordan Journal of Biological Sciences, 4,* 141-146.

Kämpfer, P., Huber, B., Thummes, K., Grün-Wollny, I. & Busse, H. J. (2007). *Int J Syst Evol Microbiol, 57,* 721-4.

Kämpfer, P. (2006). *The Prokaryotes*: Volume 3: Archaea. Bacteria: Firmicutes, Actinomycetes. In M. Dworkin et al., eds. New York, NY: Springer New York, pp. 538–604.

Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F. & Hopwood, D. A. (2000). *Practical Streptomyces Genetics*. The John Innes Foundation, Norwich, England

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H., Yi, H., Won, S. & Chun, J. (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol, 62,* 716-721.

Klykleung, N., Tanasupawat, S., Pittayakhajonwut, P., Ohkuma, M. & Kudo T. (2015). *Amycolatopsis stemonae* sp. nov., isolated from a Thai medicinal plant. *Int J Syst Evol Microbiol, 65,* 3894-3899.

Kruasuwan, W., Salih, T. S,, Brozio, S., Hoskisson, P. A. & Thamchaipenet A. (2017). Draft genome sequence of plant growth-promoting endophytic *Streptomyces* sp. GKU 895 isolated from the roots of sugarcane. *Genome Announc, 5,* pp.e00358-17.

Lazzarini, A., Cavaletti, L., Toppo, G. & Marinelli, F. (2000). Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek, 78,* 399-405.

Matsumoto, A., Takahashi, Y., Kudo, T., Seino, A., Iwai, Y. & Omura, S. (2000). *Actinoplanes capillaceus* sp. nov., a new species of the genus Actinoplanes. *Antonie van Leeuwenhoek, 78,* 107-115.

Ngaemthao, W., Chunhametha, S. & Suriyachadkun, C. (2016). *Actinoplanes subglobosus* sp. nov., isolated from mixed deciduous forest soil. *International journal of systematic and evolutionary microbiology, 66,* 4850-4855.

Nie, G. X., Ming, H., Li, S., Zhou, E. M., Cheng, J., Tang, X., Feng, H. G., Tang, S. K. & Li, W. J. (2012). *Amycolatopsis dongchuanensis* sp. nov., an actinobacterium isolated from soil. *Int J Syst Evol Microbiol, 62,* 2650-6.

Parte, A. C. (2018). LPSN – List of Prokaryotic names with Standing in Nomenclature (bacterio.net), 20 years on. *International Journal of Systematic and Evolutionary Microbiology, 68,*1825-1829.

Peng, G., Xiong, D. S., Li, L. C., Hu, J. Y., Bao, S., Chen, Y. W., Li, Y. Q., Xu, L. H., Miao, C. P. & Zhao, L. X. (2019). *Amycolatopsis panacis* sp. nov., isolated from Panax notoginseng rhizospheric soil. *Int J Syst Evol Microbiol, 69,* 567-571.

Qin, S., Li, J., Chen, H. H., Zhao, G. Z., Zhu, W. Y, Jiang, C. L., Xu, L. H. & Li, W. J. (2009). Isolation, Diversity, and Antimicrobial Activity of Rare Actinobacteri from Medicinal Plants of Tropical Rain Forests in Xishuangbanna, China. *Applied and Environmental Microbiology, 75,* 6176-6186.

Saintpierre-Bonaccio, D., Amir, H., Pineau, R., Tan, G. A. & Goodfellow, M. (2005). *Amycolatopsis plumensis* sp. nov., a novel bioactive actinomycete isolated from a New-Caledonian

brown hypermagnesian ultramafic soil. *International journal of systematic and evolutionary microbiology*, *55,* 2057-2061.

Shen, Y., Liu, C., Wang, X., Zhao, J., Jia, F., Zhang, Y., Wang, L., Yang, D. & Xiang, W. (2013). *Actinoplanes hulinensis* sp. nov., a novel actinomycete isolated from soybean root (Glycine max (L.) Merr). *Antonie van Leeuwenhoek, 103,* 293-298.

Shephered, M. D., Kharel, M. K., Bosserman, & M. A., Rohr, J. (2010). Laboratory Maintenance of *Streptomyces* species. *Current Protocols in Microbiology*, *10*, 1-10.

Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic and Evolutionary Microbiology*, *16,* 313-340.

Singh, H., Naik, B., Kumar, V. & Bisht, G. S. (2018). Screening of endophytic actinomycetes for their herbicidal activity. *Annals of Agrarian Science*, *16,* 101-107.

Souza, W. R., Silva, R. E., Goodfellow, M,. Busarakam, K., Figueiro, F. S., Ferreira, D., Rodrigues-Filho, E., Moraes, L. A. & Zucchi, T. D. (2015). *Amycolatopsis rhabdoformis* sp. nov., an actinomycete isolated from a tropical forest soil. *Int J Syst Evol Microbiol*, *65*, 1786-1793.

Sun, W., Dong, G. .X, Zhang, Y. Q., Wei, Y. Z., Li, Q. P., Yu, L. Y., Klenk, H. P. & Zhang, Y. Q. (2009). Actinoplanes sichuanensis sp. nov. and *Actinoplanes xinjiangensis* sp. nov. *Int J Syst Evol Microbiol*, *59*, 2763-2768.

Tan, G.Y., Ward, A. C. & Goodfellow M. 2006. Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. *Systematic and Applied Microbiology*, *29*, 557–569.

Tang, S. K., Wang, Y., Guan, T. W., Lee, J. C., Kim, C. J. & Li, W. J. (2010). *Amycolatopsis halophila* sp. nov., a halophilic actinomycete isolated from a salt lake. *Int J Syst Evol Microbiol*, *60,* 1073-1081.

Tiwari, K. & Gupta, R. K. (2013). Diversity and isolation of rare actinomycetes: an overview. *Crit Rev Microbiol*, *39,* 256–294.

Wink, J. M., Kroppenstedt, R. M, Ganguli, B.N., Nadkarni, S. R., Schumann. P, Seibert, G. & Stackebrandt, E. (2003). Three new antibiotic producing species of the genus Amycolatopsis, *Amycolatopsis balhimycina* sp. nov., *A. tolypomycina* sp. nov., *A. vancoresmycina* sp. nov., and description of *Amycolatopsis keratiniphila* subsp. keratiniphila subsp. nov. and *A. keratiniphila* subsp. nogabecina subsp. nov. *Systematic and applied microbiology*, *26,* 38-46.

Zucchi, T. D., Bonda, A. N., Frank, S., Kim, B. Y., Kshetrimayum, J. D. & Goodfellow, M. (2012). *Amycolatopsis bartoniae* sp. nov. and *Amycolatopsis bullii* sp. nov., mesophilic actinomycetes isolated from arid Australian soils. *Antonie van Leeuwenhoek*, *102,* 91-98.